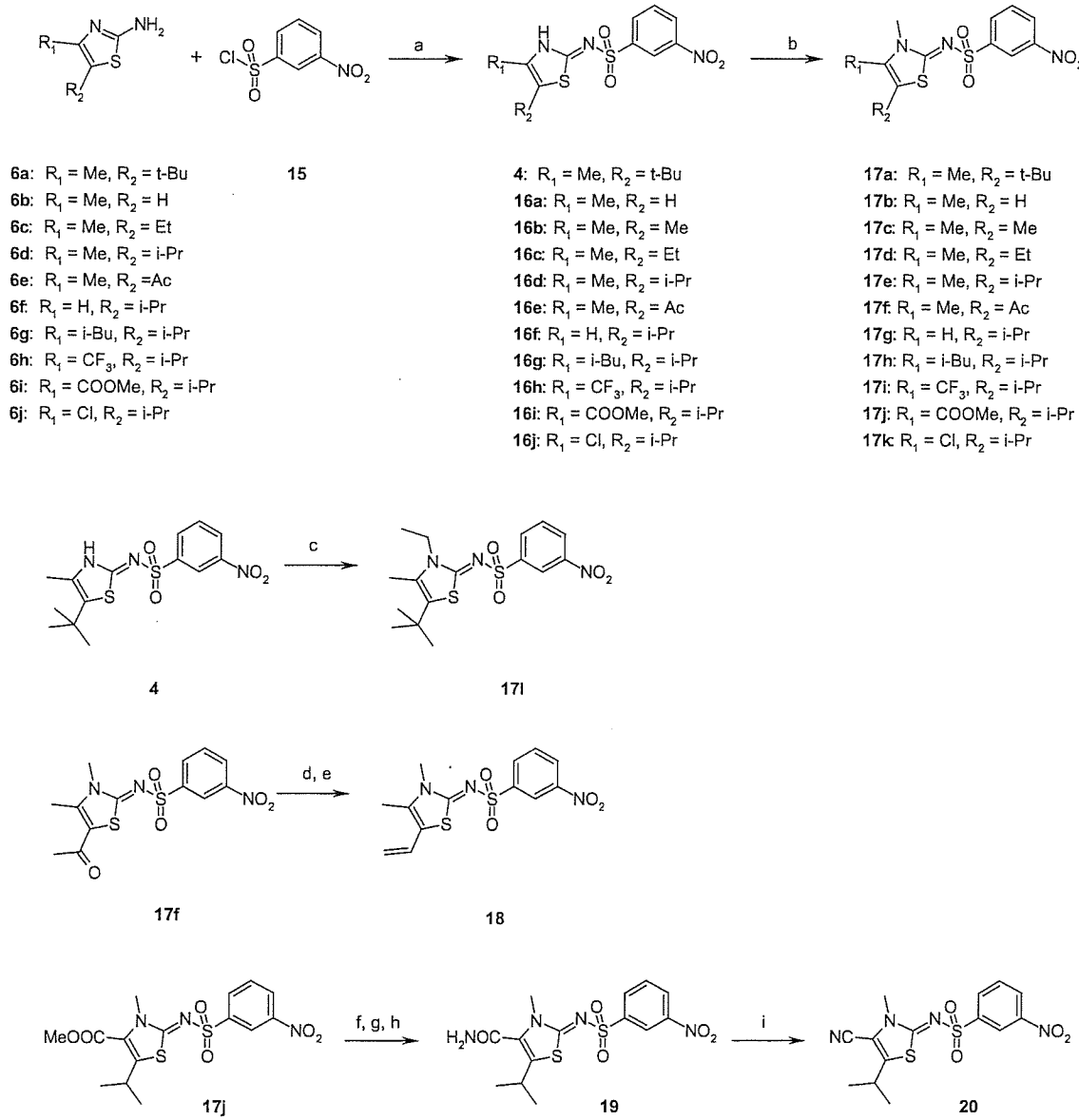
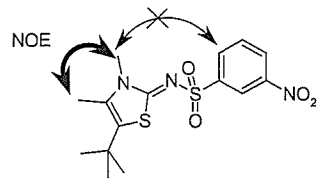
aminothiazole **6h** was synthesized from trifluoroacetoacetic acid ethyl ester **7** in five steps. The iodine-mediated cyclization of ester **7** with thiourea in isopropanol and the protection of the resultant amine with Boc anhydride provided compound **8**. The bis-alkylation of **8** using methyllithium provided 5-hydroxymethylethyl-2-aminothiazole **9**. The treatment of **9** with triethylsilane and trifluoroacetic acid effectively removed the tertiary hydroxy group and the Boc group, respectively, to afford the desired 4-trifluoromethyl-2-aminothiazole **6h**. 4-Chloro-2-aminothiazole **6j** was synthesized from chloroacetic acid **10** in six steps. The cyclization of chloroacetic acid **10** with thiourea, and subsequent formylation and chlorination, resulted in 5-formylthiazole **11**.[12] Treatment of **11** with Boc_2O, followed by oxidation and esterification, afforded the methyl ester **13**. Treatment of compound **13** with methyllithium, then triethylsilane and trifluoroacetic acid afforded the aminothiazole **6j**.

The 2-aminothiazoles **6a–j** were reacted with 3-nitrobenzenesulfonylchloride (**15**) to afford the thiazolidenebenzenesulfonamide derivatives **4** and **16a–j** (Scheme 2). Methylation of **4** and **16a–j** using methyl iodide in the presence of sodium hydride afforded the thiazolidenesulfonamides **17a–k**. Compound **4** was also converted into the corresponding *N*-ethyl derivative **17l** in a similar manner employing ethyl iodide. Alkylation of the nitrogen at the 3-position in **17a–l** was confirmed by NMR (Fig. 2). A nuclear Overhauser effect (NOE) was observed between the 3-methyl protons and the 4-methyl protons, but not between the 3-methyl protons



Scheme 2. Reagents: (a) Py; (b) MeI, NaH/THF; (c) EtI, NaH/THF; (d) $NaBH_4$/EtOH; (e) MsCl, Et_3N/CH_2Cl_2; (f) NH_3aq/THF; (g) $SOCl_2$; (h) NH_3aq/$CHCl_3$; (i) $POCl_3$, DMF/1,2-dichloroethane.



17a

Figure 2. Confirmation of the structure of **17a**. An NOE was observed between the 3-methyl and 4-methyl protons on the thiazole ring, whereas no NOE signal was observed between the 3-methyl proton on the thiazole ring and the phenyl ring.

and aromatic protons, therefore the alkylated position was assigned to the ring nitrogen. Treatment of ketone **17f** with sodium borohydride and dehydration of the resulting alcohol generated vinyl derivative **18**. Hydrolysis of **17j** and subsequent amidation afforded carboxamide **19**, which on dehydration afforded nitrile **20**.

3. Results and discussion

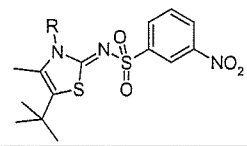
In order to identify the compounds that exhibit anti-HIV-1 activity against the Y181C mutant of HIV-1, we conducted a large-scale high-throughput screening (HTS) of in-house compound libraries in MT-4 cells acutely infected with HIV-$1_{IIIB\text{-}R}$. HIV-$1_{IIIB\text{-}R}$ is a nevirapine- and MKC-442-resistant HIV-1 strain, having the Y181C mutation. Our screening identified compound **4** as a primary hit, which possessed good anti-HIV-1 activity against both HIV-1_{IIIB} and HIV-$1_{IIIB\text{-}R}$ with EC_{50} values of 0.50 and 0.79 μM, respectively (data not shown). Compound **4** inhibited WT, Y181C, and K103N RTs with IC_{50} values of 0.37, 0.47, and 32 μM, respectively (Table 1).

In order to increase the inhibitory activity against WT, Y181C, and K103N RTs as well as HIV-1 replication, we synthesized a series of compound **4** analogues and evaluated for their inhibitory effects on these enzymes and HIV-1 replication in acutely infected MT-4 cells (Tables 1 and 2).

Previous studies have shown that two aromatic systems arranged in a 'butterfly-like orientation' are required for the inhibition of RT enzymes.[13,14] We therefore attempted to alkylate the ring nitrogen on the thiazole ring of compound **4** in order to confer a 'butterfly-like orientation' on the conformation of **4**. The introduction of the methyl group at the 3-position on the thiazole ring, compound **17a**, resulted in a 7- and 2.5-fold increase of the activity against Y181C and K103N RTs, respectively, when compared to unsubstituted compound **4**. In contrast, the introduction of an ethyl group (compound **17l**) at the 3-position significantly reduced the activity against the enzymes, confirming that the methyl group at the 3-position is crucial to the inhibition of these RTs. X-ray crystallography of **17a** revealed that the 3-methylated thiazolidenesulfonamide framework had a (*Z*)-thiazolidene conformation and provided a 'butterfly-like' structure in the two aromatic systems, as observed in other NNRTIs (Fig. 3).[13,14] In the case of **17a**, one oxygen atom of the sulfonyl group existed in the same plane of the phenyl ring, and the other existed in the plane on the thiazole ring. As a result, the RT inhibition was enhanced by the rigid conformation of the 'butterfly-like' (*Z*)-structure derived from methylation at the 3-position on the thiazole ring. Although the activity of **17a** against the WT RT was similar to that of **4**, the anti-HIV-1 activity of **17a** was 6-fold stronger than that of **4**. Consequently, the TI value of **17a** exceeded 290. Additionally, the introduction of the methyl group may be responsible for better cell permeability or compound stability under assay conditions.

We next investigated the effect of the substituents at the 5-position on the thiazole ring. As shown in Table 2, among the compounds possessing a methyl group at the 4-position, compounds **17a**, **17c–e**, and **18** retained the inhibitory activity against the WT RT. However, the introduction of an acetyl group at the 5-position (compound **17f**) led to a dramatic loss of the activity. Substitution with bulkier alkyl groups such as *tert*-butyl and isopropyl (compounds **17a** and **17e**; IC_{50} values of 0.066 and 0.071 μM, respectively) exhibited a superior activity against the Y181C RT but not against the WT or K103N RT: therefore the activity against the

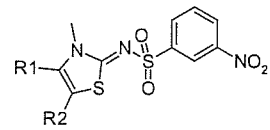
Table 1. In vitro activity of 3-substituted thiazolidene derivatives



Compounds	R	IC_{50} (μM)[a]			EC_{50} (μM)[b]	CC_{50} (μM)[c]	TI[d]
		WT	K103N	Y181C			
4	H	0.37	32	0.47	0.50	>25	>50
17a	Me	0.27	13	0.066	0.085	>25	>290
17l	Et	6.0	>50	0.12	>11.5	11.5	<1

[a] Compound concentration required to achieve 50% inhibition of recombinant HIV-1 RT activity.
[b] Compound concentration required to achieve 50% protection of MT-4 cells from the HIV-1 induced CPE as determined by the MTT method.
[c] Compound concentration required to reduce the viability of mock-infected MT-4 cells as determined by the MTT method.
[d] Therapeutic index (CC_{50}/EC_{50}).

Table 2. In vitro activity of 4- and 5-substituted thiazolidene derivatives



Compounds	R_1	R_2	IC_{50} (μM)[a]			EC_{50} (μM)[b]	CC_{50} (μM)[c]	TI[d]
			WT	K103N	Y181C			
4			0.37	32	0.47	0.50	>25	>50
17a	Me	*t*-Bu	0.27	13	0.066	0.085	>25	>290
17b	Me	H	>50	>50	>50	>25	>25	—
17c	Me	Me	0.97	>50	33	1.1	>25	>23
17d	Me	Et	0.85	6.3	0.47	0.23	>25	>110
17e	Me	*i*-Pr	0.34	20	0.071	0.10	>25	>250
17f	Me	Ac	>50	>50	>50	>25	>25	—
17g	H	*i*-Pr	0.60	>50	0.70	3.1	>25	>8
17h	*i*-Bu	*i*-Pr	>50	>50	>25	>21.4	21.4	—
17i	CF_3	*i*-Pr	0.60	42	0.26	0.26	>25	>96
17j	COOMe	*i*-Pr	>50	>50	>25	>25	>25	—
17k	Cl	*i*-Pr	0.077	6.9	0.13	0.048	24	500
18	Me	$CH{=}CH_2$	0.15	6.2	9.6	0.097	4.4	45
19	$CONH_2$	*i*-Pr	39	>50	>25	>25	>25	—
20	CN	*i*-Pr	0.62	>50	0.64	0.71	>25	>35
Nevirapine (**1**)			0.0026	1.1	>1.9	0.0053	>25	>4700
Delavirdine (**2**)			0.042	4.8	7.5	0.0039	>25	>6400
Efavirenz (**3**)			0.0069	0.021	0.0040	0.0027	8.5	3200

[a–d]See footnotes in Table 1.

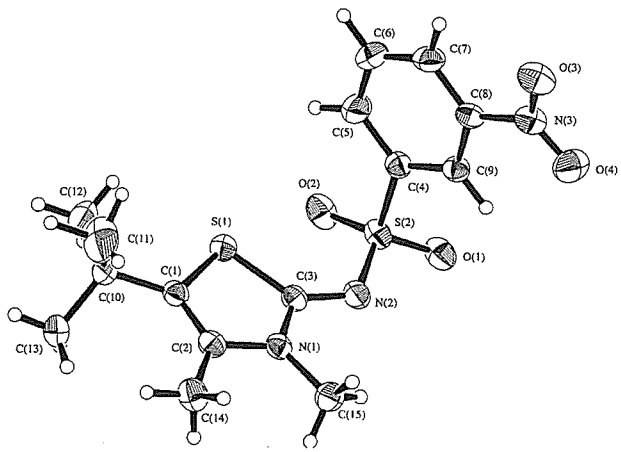


Figure 3. X-ray crystallography of compound **17a**.

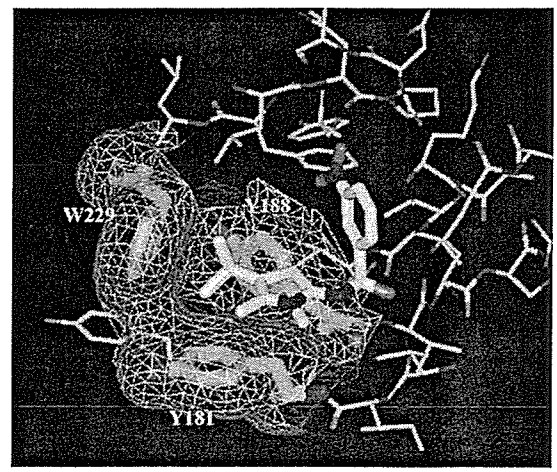


Figure 4. Docking study of **17a** within RT nonnucleoside binding site.

Y181C RT is affected by the nature of 5-position substituents. These results also suggest that the alkyl group at the 5-position exists near the Y181 of the HIV-1 RT. In the docking study of **17a** with a RT nonnucleoside binding site, it was predicted that the *tert*-butyl group would occupy the hydrophobic pocket constructed by Y181, Y188, and W229 and that it was positioned near the Y181 (Fig. 4). Therefore, the bulky alkyl groups at the 5-position on the thiazole ring seem to be crucial for the inhibition of the Y181C RT. With respect to the substituents at the 5-position on the thiazole ring, both ethyl (**17d**) and vinyl (**18**) groups improved the inhibitory activity against the K103N RT, and **17d** and **18** were significantly more potent than **4**. The K103N mutation reduced sensitivity to nevirapine and delavirdine 423- and 114-fold, respectively, but compounds **17a**, **17d**, and **18** were only 48-, 7-, and 41-fold less potent against the K103N mutant, respectively. Compounds **17a**, **17d**, **17e**, and **18** showed potent anti-HIV-1 activity with EC_{50} values of 0.085, 0.23, 0.10, and 0.097 μM, respectively. For each compound, its EC_{50} value was strongly associated with its IC_{50} value for WT RT.

In order to find more potent inhibitors, further modifications at the 4-position on the thiazole ring were performed. Among the 5-isopropyl derivatives prepared,

those with sterically small substituents at the 4-position exhibited the strong activity against both of the WT and the Y181C RTs: no apparent preference for either electron-withdrawing or electron-donating substituents was observed. Those compounds with methyl (**17e**), trifluoromethyl (**17i**), and cyano (**20**) substituents at the 4-position possessed the inhibitory activity similar to that of the unsubstituted compound (**17g**). However, the compounds with a bulkier *iso*-butyl group (**17h**) or hydrophilic groups, such as methoxycarbonyl (**17j**) and carbamoyl (**19**), showed a lack of RT inhibition. The 4-methyl derivative (**17e**) showed the strongest activity against the Y181C RT with an IC_{50} value of 0.071 μM, which was five times greater than its activity against the WT RT (IC_{50}: 0.34 μM). In contrast to **17e**, the 4-chloro derivative (**17k**) showed a stronger activity against the WT RT with an IC_{50} value of 0.077 μM, and this value was about twice stronger than against the Y181C RT (IC_{50}: 0.13 μM). The introduction of a chloro group to the 4-position (**17k**) also improved the activity against K103N RT (IC_{50}: 6.9 μM) when compared to **17e**. Among the 5-isopropyl derivatives, trifluoromethyl (**17i**), chloro (**17k**), and cyano derivatives (**20**) possessed potent anti-HIV-1 activity with EC_{50} values of 0.26, 0.048, and 0.71 μM, respectively. The SAR of their anti-HIV-1 activity also correlated well with the SAR deduced from the inhibitory activity against WT RT.

Taken together, three factors affect the activity against HIV-1 RT in this series. First, the 'butterfly-like' structure of the thiazolidenebenzenesulfonamide is crucial for RT inhibition. Second, the inclusion of bulky substituents, such as *tert*-butyl and isopropyl, at the 5-position on the thiazole ring enhances the inhibitory activity against the Y181C RT. Third, substituents with an appropriate size at the 4-position on the thiazole ring, such as methyl and chloro groups, are necessary for RT inhibition.

Since there was a good correlation between the EC_{50} values for HIV-1 replication and the IC_{50} values for the WT RT, we concluded that compound **4** and its related compounds were NNRTIs. Among them, the 5-*tert*-butyl-4-methyl (**17a**) and 4-chloro-5-isopropyl analogues (**17k**) showed the most potent anti-HIV-1 activity with EC_{50} values of 0.085 and 0.048 μM, respectively, which represent 6–10-fold improvements over the primary hit compound **4**, while still retaining good TI values.

These results present a significant springboard for the further improvement of the activity spectrum of thiazolidenebenzenesulfonamide analogues as novel NNRTIs.

4. Conclusion

The HTS of an in-house compound library in MT-4 cells acutely infected with HIV-$1_{IIIB\text{-}R}$ has been successfully applied to the discovery of a novel class of NNRTIs, which have potent inhibitory activity against both WT and Y181C RT. The SAR analysis of these thiazolidenebenzenesulfonamide derivatives indicates that the 'butterfly-like' conformation derived from the sulfonamide structure is important for the activity against the WT RT and that the methyl substituent at the 3-position on the thiazole ring is crucial for the fixed configuration to enhance the activity. The inclusion of hydrophobic bulky substituents at the 5-position on the thiazole ring is favorable for enhanced activity against the Y181C RT. Among the newly synthesized compounds, both **17a** and **17k** potently inhibit HIV-1 replication with EC_{50} values of 0.085 and 0.048 μM, respectively. They also have a potent activity against the WT and Y181C RT and to a lesser extent against the K103N RT. The thiazolidenebenzenesulfonamide analogues provide valuable leads for the discovery of the next generation NNRTIs.

5. Experimental

5.1. Chemistry

Melting points were determined on a Yanaco micromelting apparatus or Büchi B-545 melting point apparatus and are uncorrected. Proton magnetic resonance (^1H NMR) spectra were obtained in $CDCl_3$ or dimethylsulfoxide-d_6 (DMSO-d_6) using a JEOL JNM-EX90, JNM-EX400, JNM-GX500, or JNM-A500 spectrometer. Chemical shifts were expressed in δ (ppm) values with tetramethylsilane as an internal standard (in NMR description, s: singlet, d: doublet, t: triplet, m: multiplet, br: broad peak). Mass spectra (MS) were recorded on a JEOL JMS-DX300 or a HITACHI M-80 mass spectrometer. Elemental analysis was carried out on Yanaco MT-3 or MT-5 CHN analyzer and a Yokogawa IC7000S Ion Chromatoanalyzer. Chromatographic separations were performed using a silica gel column (Merck Kieselgel 60). Analytical thin-layer chromatography (TLC) was carried out on precoated glass plates (Merck Kieselgel 60F254). The structure of target compounds were deduced from mass spectral and ^1H NMR data, and were verified by a single crystal X-ray structure determination for compound **17a** (Fig. 3).

The following known materials were prepared as described in the literature: **6a**,[15] **6f**,[16] **6i**,[17] or obtained from commercial suppliers: **6b**, **15**.

5.1.1. 5-Ethyl-4-methylthiazole-2-amine (6c). To a stirred solution of 2-butanone (**5a**, 5.00 g, 58.1 mmol) and bromotrimethylsilane (8.3 mL, 63.9 mmol) in acetonitrile (50 mL) was added slowly dropwise dimethylsulfoxide (4.5 mL, 63.9 mmol) under ice-bath cooling. After stirring for 15 min, the solution was warmed to room temperature and stirred for 2 h. The reaction mixture was poured into ice water and extracted with diethyl ether. The organic layer was washed with brine, then dried over anhydrous sodium sulfate, and evaporated under reduced pressure. Ethanol (50 mL) and thiourea (4.42 g, 58.1 mmol) was added to the residue and refluxed for 1 h. The mixture was cooled to room temperature, and the resulting precipitate was collected by filtration. The precipitate was washed with diethyl ether

and hexane to give **6c** hydrobromide (2.10 g, 15%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ: 1.10 (3H, t, J = 7.5 Hz, CH_3 of *t*-Bu), 2.11 (3H, s, CH_3 of Et), 2.58 (2H, q, J = 7.5 Hz, CH_2 of Et), 9.15 (2H, br s, NH_2); FAB-MS *m/z*: 143 (M^++1).

The following compounds were obtained in the same manner.

5.1.2. 4-Methyl-5-isopropylthiazole-2-amine (6d). 57% yield; ^{1}H NMR ($CDCl_3$) δ: 1.11 (6H, d, J = 6.8 Hz, CH_3 of *i*-Pr), 1.97 (3H, s, 4-Me), 3.02 (1H, heptet, J = 6.8 Hz, CH of *i*-Pr), 6.49 (2H, br s, NH_2); FAB-MS *m/z*: 157 (M^++1).

5.1.3. 1-(2-Amino-4-methyl-1,3-thiazol-5-yl)ethanone (6e). 84% yield; ^{1}H NMR (DMSO-d_6) δ: 2.44 (3H, s, CH_3 of Ac), 2.52 (3H, s, 4-Me), 9.80 (2H, br s, NH_2); FAB-MS *m/z*: 157 (M^++1).

5.1.4. 4-Isobutyl-5-isopropylthiazole-2-amine (6g). 44% yield; ^{1}H NMR (DMSO-d_6) δ: 0.85 (6H, d, J = 6.8 Hz, CH_3 of *i*-Bu), 1.12 (6H, d, J = 6.8 Hz, CH_3 of *i*-Pr), 1.89 (1H, m, CH of *i*-Bu), 2.19 (2H, d, J = 6.8 Hz, CH_2 of *i*-Bu), 3.03 (1H, heptet, J = 6.8 Hz, CH of *i*-Pr), 6.50 (2H, br s, NH_2); FAB-MS *m/z*: 199 (M^++1).

5.1.5. Ethyl 2-[(*tert*-butoxycarbonyl)amino]-4-trifluoromethyl-1,3-thiazole-5-carboxylate (8). To a stirred mixture of ethyl trifluoroacetoacetate (7.36 g, 60 mmol), thiourea (13.68 g, 180 mmol), and isopropanol (30 mL) was added iodine (11.42 g, 90.0 mmol) and the mixture was refluxed for 3 h. The reaction mixture was concentrated under reduced pressure, and the residue was recrystallized from methanol–water. To a tetrahydrofuran (70 mL) solution of the precipitate, di-*tert*-butyl dicarbonate (7.61 g, 34.9 mmol), and *N*,*N*-dimethylaminopyridine (178 mg, 1.46 mmol) was added. Then the mixture was stirred at 60 °C for 1 h. The reaction mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (ethyl acetate–toluene) to give **7** (10.43 g, 49%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 1.55 (9H, s, CH_3 of *t*-Bu), 1.57 (3H, t, J = 7.1 Hz, CH_3 of Et), 4.36 (2H, q, J = 7.1 Hz, CH_2 of Et), 8.48 (2H, br s, NH_2); FAB-MS *m/z*: 341 (M^++1).

5.1.6. *tert*-Butyl [5-(1-hydroxy-1-methylethyl)-4-trifluoromethyl-1,3-thiazol-2-yl]carbamate (9). Under argon atmosphere, to a tetrahydrofuran (200 mL) solution of **8** (4.25 g, 12.5 mmol) was added dropwise 1.14 M methyllithium diethyl ether solution (44 mL, 50 mmol) at −78 °C and the solution was stirred at the same temperature for 0.5 h. The reaction mixture was quenched with saturated aqueous ammonium chloride solution and extracted with ethyl acetate. The organic layer was washed with brine, and then dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (ethyl acetate–hexane) to give **9** (2.97 g, 73%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 1.53 (9H, s, CH_3 of *t*-Bu), 1.71 (6H, s, Me), 2.43 (1H, s, OH), 8.36 (1H, br s, NH); FAB-MS *m/z*: 327 (M^++1).

5.1.7. 5-Isopropyl-4-trifluoromethyl-1,3-thiazol-2-amine (6h). To a stirred solution of **9** (2.97 g, 9.11 mmol) in trifluoroacetic acid (40 mL) was added triethylsilane (4.35 mL, 27.3 mmol) and stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (ethyl acetate–hexane) to give **6h** (2.20 g, quantitative) as colorless syrup. ^{1}H NMR ($CDCl_3$) δ: 1.30 (6H, d, J = 6.8 Hz, CH_3 of *i*-Pr), 3.46 (1H, heptet, J = 6.8 Hz, CH of *i*-Pr), 7.06 (2H, br s, NH_2); FAB-MS *m/z*: 211 (M^++1).

5.1.8. 2-Amino-4-chloro-1,3-thiazole-5-carbaldehyde (11). To a stirred solution of thiourea (100 g, 1.31 mol) in *N*,*N*-dimethylformamide (1.0 L) was added portionwise chloroacetic acid (124 g, 1.31 mol) and stirred at 40 °C for 2 h. To the stirred reaction mixture under ice-bath cooling was added dropwise phosphorous oxychloride (424 mL, 4.59 mol) for 1 h. The mixture was stirred at 60 °C for 30 min, and then stirred at 90 °C for 5 h. The reaction mixture was poured into ice-water and sodium chloride (456 g, 10.2 mol), calcium hydroxide (336 g, 5.93 mol) was added. The resulting precipitate was collected by filtration and washed with water until the filtrate was neutral. The precipitate was dried under reduced pressure to give **11** (165 g, 72%) as pink solid. Two tautomers were observed on ^{1}H NMR spectrum. ^{1}H NMR (DMSO-d_6) one tautomer: δ: 8.70 (1H, s, NH_2), 9.92 (1H, s, CHO), 13.20 (1H, br s, NH_2) and the other tautomer: 8.74 (2H, s, NH_2), 9.64 (1H, s, CHO); FAB-MS *m/z*: 237 (M^++1).

5.1.9. *tert*-Butyl (4-chloro-5-formyl-1,3-thiazol-2-yl)carbamate (12). To a stirred solution of **11** (34.4 g, 0.212 mol) in 1,4-dioxane (500 mL) was added di-*tert*-butyl dicarbonate (55.4 g, 0.254 mol) and *N*,*N*-dimethylaminopyridine (2.58 g, 0.021 mol). The reaction mixture was stirred at 60 °C for 2 h. The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate. The solution was washed with 5% aqueous potassium hydrogen sulfate solution and brine, and then dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to give **12** (48.2 g, 87%) as a brown solid. ^{1}H NMR (DMSO-d_6) δ: 1.50 (9H, s, CH_3 of *t*-Bu), 9.86 (1H, s, CHO), 12.53 (1H, s, NH); FAB-MS *m/z*: 263 (M^++1).

5.1.10. Methyl 2-[(*tert*-butoxycarbonyl)amino]-4-chloro-1,3-thiazole-5-carboxylate (13). A stirred mixture of **12** (30.0 g, 114 mmol), potassium dihydrogensulfate (46.5 g, 342 mmol) solution in water (200 mL) and 2-methyl-2-butene (157 mL, 1.48 mol) in *tert*-butanol (1.20 L) was added dropwise sodium chlorite (61.9 g, 684 mmol) in water (120 mL) under ice-bath cooling. The mixture was stirred at room temperature for 6 h. To the reaction mixture, ethyl acetate was added and washed with 5% aqueous potassium hydrogen sulfate solution. The organic layer was alkalinized with 1 M aqueous sodium hydroxide solution and washed with ethyl acetate. The aqueous layer was acidified to pH 3 with potassium hydrogen sulfate and extracted with chloroform. The organic layer was dried over sodium sulfate and evaporated. The residue was dissolved in

methanol (150mL) and *N*,*N*-dimethylformamide (300mL). To the solution, 1-hydroxybenzotriazole (23.1g, 171mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (54.6g, 285mmol), and *N*,*N*-dimethylaminopyridine (134mg, 1.1mmol) was added and stirred at room temperature for 10h. The solvent was removed under reduced pressure and 10% aqueous citric acid solution was added. The mixture was extracted with ethyl acetate–toluene and washed with water, saturated aqueous sodium hydrogen carbonate solution and brine. The organic layer was dried over sodium sulfate and evaporated to give **13** (19.0g, 57%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ: 1.50 (9H, s, CH_3 of *t*-Bu), 3.79 (3H, s, CH_3 of COOMe), 12.30 (1H, s, NH); FAB-MS *m/z*: 293 (M^++1).

5.1.11. *tert*-Butyl [4-chloro-5-(1-hydroxy-1-methylethyl)-1,3-thiazol-2-yl]carbamate (14). Compound **14** was obtained from **13** in the same manner as described in the synthesis of **9**. 98% yield as a colorless solid. ^{1}H NMR (DMSO-d_6) δ: 1.47 (9H, s, CH_3 of *t*-Bu), 1.53 (6H, s, Me), 5.82 (1H, s, OH), 11.41 (1H, s, NH); FAB-MS *m/z*: 293 (M^++1).

5.1.12. 4-Chloro-5-isopropyl-1,3-thiazol-2-amine (6j). To a stirred solution of **14** (20g, 6.82mmol) in trifluoroacetic acid (20mL) was added triethylsilane (10mL) and stirred at room temperature for 1h. The mixture was evaporated, saturated aqueous sodium hydrogen carbonate solution was added and extracted with ethyl acetate. The organic layer was dried over sodium sulfate and evaporated. The residue was purified by silica gel column chromatography (ethyl acetate–hexane) to give **6j** (1.17g, 97%) as a red solid. ^{1}H NMR (DMSO-d_6) δ: 1.14 (6H, d, J = 6.9Hz, CH_3 of *i*-Pr), 3.05 (1H, heptet, J = 6.9Hz, CH of *i*-Pr), 7.20 (2H, br s, NH_2); FAB-MS *m/z*: 177 (M^++1).

5.1.13. *N*-(5-*tert*-Butyl-4-methyl-1,3-thiazol-2-yl)-3-nitrobenzenesulfonamide (4). To a solution of **6a** (5.88g, 34.6mmol) in pyridine (200mL) was added **15** (9.46g, 42.7mmol) and the solution was stirred at room temperature for 12h. The reaction mixture was poured into water and extracted with chloroform. The organic layer was washed with saturated aqueous sodium hydrogen carbonate solution, 1M hydrochloric acid, and brine and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (methanol–chloroform) and recrystallized from methanol to give **4** (8.49g, 69%) as a yellow crystals. Mp 212–213°C. ^{1}H NMR ($CDCl_3$) δ: 1.30 (9H, s, CH_3 of *t*-Bu), 2.16 (3H, s, 4-Me), 7.86 (1H, t, J = 8.3Hz, benzene), 8.21 (1H, dd, J = 2.0, 8.3Hz, benzene), 8.42 (1H, ddd, J = 1.8, 2.0, 8.3Hz, benzene), 8.46 (1H, t, J = 1.8Hz, benzene), 12.58 (1H, br s, NH); FAB-MS *m/z*: 356 (M^++1). Anal. Calcd for $C_{14}H_{17}N_3O_4S_2$: C, 47.31; H, 4.82; N, 11.82; S, 18.04. Found: C, 47.06; H, 4.84; N, 11.80; S, 17.84.

The following compounds were obtained in the same manner.

5.1.14. *N*-(4-Methyl-1,3-thiazol-2-yl)-3-nitrobenzenesulfonamide (16a). 60% yield; ^{1}H NMR (DMSO-d_6) δ: 2.10 (3H, s, Me), 6.48 (1H, s, thiazole), 7.85 (1H, t, J = 7.8Hz, benzene), 8.22 (1H, ddd, J = 1.0, 2.0, 7.8Hz, benzene), 8.43 (1H, ddd, J = 1.0, 1.5, 7.8Hz, benzene), 8.51 (1H, dd, J = 1.5, 2.0Hz, benzene), 12.88 (1H, br s, NH); FAB-MS *m/z*: 300 (M^++1).

5.1.15. *N*-(4,5-Dimethyl-1,3-thiazol-2-yl)-3-nitrobenzenesulfonamide (16b). 60% yield; ^{1}H NMR (DMSO-d_6) δ: 2.01 (3H, s, 4-Me), 2.10 (3H, s, 5-Me), 7.85 (1H, t, J = 7.8Hz, benzene), 8.20 (1H, br d, J = 7.8Hz, benzene), 8.42 (1H, ddd, J = 1.0, 2.0, 7.8Hz, benzene), 8.46 (1H, t, J = 2.0Hz, benzene), 12.63 (1H, br s, NH); FAB-MS *m/z*: 314 (M^++1).

5.1.16. *N*-(5-Ethyl-4-methyl-1,3-thiazol-2-yl)-3-nitrobenzenesulfonamide (16c). 11% yield; ^{1}H NMR (DMSO-d_6) δ: 1.09 (3H, t, J = 7.7Hz, CH_3 of Et), 2.03 (3H, s, 4-Me), 2.50 (2H, q, J = 7.7Hz, CH_2 of Et), 7.83 (1H, m, benzene), 8.22 (1H, dt, J = 1.5, 8.0Hz, benzene), 8.42 (1H, m, benzene), 8.46 (1H, m, NH); FAB-MS *m/z*: 328 (M^++1).

5.1.17. *N*-(5-Isopropyl-4-methyl-1,3-thiazol-2-yl)-3-nitrobenzenesulfonamide (16d). 32% yield; ^{1}H NMR (DMSO-d_6) δ: 1.14 (6H, d, J = 6.8Hz, CH_3 of *i*-Pr), 2.04 (3H, s, 4-Me), 3.10 (1H, heptet, J = 6.8Hz, CH of *i*-Pr), 7.85 (1H, dt, J = 0.9, 8.1Hz, benzene), 8.22 (1H, dt, J = 1.4, 7.7Hz, benzene), 8.43 (1H, m, benzene), 8.46 (1H, m, NH); FAB-MS *m/z*: 342 (M^++1).

5.1.18. *N*-(5-Acetyl-4-methyl-1,3-thiazol-2(3*H*)-ylidene)-3-nitrobenzenesulfonamide (16e). 90% yield; ^{1}H NMR (DMSO-d_6) δ: 2.44 (3H, s, CH_3 of Ac), 2.46 (3H, s, 4-Me), 7.89 (1H, t, J = 8.1Hz, benzene), 8.27 (1H, dt, J = 1.4, 8.1Hz, benzene), 8.31 (1H, s, NH), 8.47 (1H, m, benzene); FAB-MS *m/z*: 342 (M^++1).

5.1.19. *N*-(5-Isopropyl-1,3-thiazol-2-yl)-3-nitrobenzenesulfonamide (16f). 51% yield; ^{1}H NMR (DMSO-d_6) δ: 1.19 (6H, d, J = 6.8Hz, CH_3 of *i*-Pr), 2.94 (1H, heptet, J = 6.8Hz, CH of *i*-Pr), 7.05 (1H, s, thiazole), 7.86 (1H, dd, J = 7.8, 8.3Hz, benzene), 8.23 (1H, br d, J = 7.8Hz, benzene), 8.42 (1H, ddd, J = 1.5, 1.9, 8.3Hz, benzene), 8.47 (1H, dd, J = 1.5, 1.9Hz, benzene), 12.71 (1H, br s, NH); FAB-MS *m/z*: 328 (M^++1).

5.1.20. *N*-(4-Isobutyl-5-isopropyl-1,3-thiazol-2-yl)-3-nitrobenzenesulfonamide (16g). 85% yield; ^{1}H NMR (DMSO-d_6) δ: 0.84 (6H, d, J = 6.3Hz, CH_3 of *i*-Bu), 1.16 (6H, d, J = 6.8Hz, CH_3 of *i*-Pr), 1.84 (1H, m, CH of *i*-Bu), 2.30 (2H, d, J = 7.3Hz, CH_2 of *i*-Bu), 3.12 (1H, heptet, J = 6.8Hz, CH of *i*-Pr), 7.86 (1H, t, J = 7.8Hz, benzene), 8.22 (1H, br d, J = 7.8Hz, benzene), 8.43 (1H, br d, J = 7.8Hz, benzene), 8.48 (1H, t, J = 2.0Hz, benzene), 12.60 (1H, br s, NH); FAB-MS *m/z*: 384 (M^++1).

5.1.21. *N*-(5-Isopropyl-4-trifluoromethyl-1,3-thiazol-2-yl)-3-nitrobenzenesulfonamide (16h). 78% yield; ^{1}H NMR ($CDCl_3$) δ: 1.14 (6H, d, J = 6.0Hz, CH_3 of *i*-Pr), 3.24 (1H, m, CH of *i*-Pr), 7.71 (1H, dt, J = 1.1,

7.1 Hz, benzene), 8.13 (1H, dd, J = 1.1, 7.1 Hz, benzene), 8.26 (1H, dd, J = 1.1, 7.1 Hz, benzene), 8.49 (1H, d, J = 1.8 Hz, benzene); FAB-MS m/z: 393 (M^+-1).

5.1.22. Methyl 5-isopropyl-2-{[(3-nitrophenyl)sulfonyl]-amino}-1,3-thiazole-4-carboxylate (16i). 75% yield; ^{1}H NMR ($CDCl_3$) δ: 1.30 (6H, d, J = 7.0 Hz, CH_3 of i-Pr), 3.95 (1H, heptet, J = 7.0 Hz, CH of i-Pr), 3.93 (3H, s, CH_3 of COOMe), 7.69 (1H, dt, J = 7.7, 8.3 Hz, benzene), 8.27 (1H, ddd, J = 1.1, 1.8, 7.7 Hz, benzene), 8.38 (1H, ddd, J = 1.1, 2.4, 8.3 Hz, benzene), 8.77 (1H, t, J = 1.8 Hz, benzene); FAB-MS m/z: 386 (M^++1).

5.1.23. *N*-(4-Chloro-5-isopropyl-1,3-thiazol-2-yl)-3-nitrobenzenesulfonamide (16j). 35% yield; ^{1}H NMR (DMSO-d_6) δ: 1.21 (6H, d, J = 6.8 Hz, CH_3 of i-Pr), 3.12 (1H, heptet, J = 6.8 Hz, CH of i-Pr), 7.52 (1H, t, J = 8.1 Hz, benzene), 8.26 (2H, d, J = 8.1 Hz, benzene), 8.69 (2H, m, benzene and NH); FAB-MS m/z: 362 (M^++1).

5.1.24. *N*-(5-*tert*-Butyl-3,4-dimethyl-1,3-thiazol-2(3*H*)-ylidene)-3-nitrobenzenesulfonamide (17a). To a solution of **4** (8.49 g, 23.9 mmol) in tetrahydrofuran (150 mL) was added sodium hydride (60% dispersion in mineral oil: 1.44 g, 35.9 mmol) and iodomethane (2.98 mL, 47.8 mmol) under ice-bath cooling. The solution was warmed to room temperature and stirred for 12 h. The reaction mixture was poured into ice water and extracted with ethyl acetate. The organic layer was washed with brine, and then dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (chloroform) and recrystallized from methanol to give **17a** (6.56 g, 74%) as a yellow crystals. Mp 143–145 °C. ^{1}H NMR (DMSO-d_6) δ: 1.32 (9H, s, CH_3 of t-Bu), 2.12 (3H, s, 4-Me), 3.60 (3H, s, 3-Me), 7.84 (1H, dd, J = 7.9, 8.3 Hz, benzene), 8.26 (1H, br d, J = 7.9 Hz, benzene), 8.41 (1H, dd, J = 2.5, 8.3 Hz, benzene), 8.49 (1H, t, J = 2.5 Hz, benzene); FAB-MS m/z: 370 (M^++1). Anal. Calcd for $C_{15}H_{19}N_3O_4S_2$: C, 48.76; H, 5.18; N, 11.37; S, 17.36. Found: C, 48.69; H, 5.25; N, 11.31; S, 17.37.

The following compounds were obtained in the same manner.

5.1.25. *N*-(3,4-Dimethyl-1,3-thiazol-2(3*H*)-ylidene)-3-nitrobenzenesulfonamide (17b). 80% yield; mp 199–200 °C (H_2O–acetonitrile), ^{1}H NMR (DMSO-d_6) δ: 2.21 (3H, s, 4-Me), 3.42 (3H, s, 3-Me), 6.63 (1H, s, thiazole), 7.85 (1H, t, J = 7.8 Hz, benzene), 8.25 (1H, br d, J = 7.8 Hz, benzene), 8.42 (1H, ddd, J = 1.0, 2.0, 8.3 Hz, benzene), 8.49 (1H, dd, J = 1.5, 2.0 Hz, benzene), FAB-MS m/z: 313 (M^+). Anal. Calcd for $C_{11}H_{11}N_3O_4S_2 \cdot 0.3H_2O$: C, 41.45; H, 3.67; N, 13.18; S, 20.12. Found: C, 41.44; H, 3.40; N, 13.36; S, 20.24.

5.1.26. *N*-(3,4,5-Trimethyl-1,3-thiazol-2(3*H*)-ylidene)-3-nitrobenzenesulfonamide (17c). 88% yield; mp 156–158 °C (diethyl ether–acetonitrile), ^{1}H NMR (DMSO-d_6) δ: 2.13 (3H, s, 4-Me), 2.16 (3H, s, 5-Me), 3.43 (3H, s, 3-Me), 7.84 (1H, dd, J = 7.8, 8.3 Hz, benzene), 8.24 (1H, br d, J = 7.8 Hz, benzene), 8.42 (1H, ddd, J = 0.9, 2.0, 8.3 Hz, benzene), 8.48 (1H, t, J = 2.0 Hz, benzene); FAB-MS m/z: 328 (M^++1). Anal. Calcd for $C_{12}H_{13}N_3O_4S_2$: C, 44.03; H, 4.00; N, 12.84; S, 19.59. Found: C, 43.84; H, 3.90; N, 12.84; S, 19.49.

5.1.27. *N*-(5-Ethyl-3,4-dimethyl-1,3-thiazol-2(3*H*)-ylidene)-3-nitrobenzenesulfonamide (17d). 54% yield; mp 159–160 °C (ethanol). ^{1}H NMR (DMSO-d_6) δ: 1.09 (3H, t, J = 7.3 Hz, CH_3 of Et), 2.15 (3H, s, 4-Me), 2.61 (1H, q, J = 7.3 Hz, CH_2 of Et), 3.43 (3H, s, 3-Me), 7.85 (1H, dd, J = 7.8, 8.3 Hz, benzene), 8.25 (1H, ddd, J = 1.0, 1.9, 7.8 Hz, benzene), 8.44 (1H, ddd, J = 1.0, 1.9, 8.3 Hz, benzene), 8.49 (1H, t, J = 1.9 Hz, benzene); FAB-MS m/z: 342 (M^++1). Anal. Calcd for $C_{13}H_{15}N_3O_4S_2$: C, 45.73; H, 4.43; N, 12.31; S, 18.78. Found: C, 45.78; H, 4.48; N, 12.45; S, 18.76.

5.1.28. *N*-(3,4-Dimethyl-5-isopropyl-1,3-thiazol-2(3*H*)-ylidene)-3-nitrobenzenesulfonamide (17e). 56% yield; mp 124–125 °C (diethyl ether–hexane). ^{1}H NMR (DMSO-d_6) δ: 1.15 (6H, d, J = 6.9 Hz, CH_3 of i-Pr), 2.17 (3H, s, 4-Me), 3.21 (1H, heptet, J = 6.9 Hz, CH of i-Pr), 3.42 (3H, s, 3-Me), 7.85 (1H, dd, J = 7.8, 8.3 Hz, benzene), 8.26 (1H, ddd, J = 1.0, 1.9, 7.8 Hz, benzene), 8.43 (1H, ddd, J = 1.0, 1.9, 8.3 Hz, benzene), 8.49 (1H, t, J = 1.9 Hz, benzene); FAB-MS m/z: 356 (M^++1). Anal. Calcd for $C_{14}H_{17}N_3O_4S_2$: C, 47.31; H, 4.82; N, 11.82; S, 18.04. Found: C, 47.27; H, 4.70; N, 11.88; S, 18.09.

5.1.29. *N*-(5-Acetyl-3,4-dimethyl-1,3-thiazol-2(3*H*)-ylidene)-3-nitrobenzenesulfonamide (17f). 66% yield; mp 205–206 °C (acetonitrile). ^{1}H NMR (DMSO-d_6) δ: 2.08 (3H, s, 4-Me), 2.48 (3H, s, CH_3 of Ac), 2.58 (3H, s, 3-Me), 7.88 (1H, t, J = 8.0 Hz, benzene), 8.30 (1H, ddd, J = 0.9, 1.8, 8.0 Hz, benzene), 8.46 (1H, ddd, 0.9, 1.8, 8.0 Hz, benzene), 8.51 (1H, t, J = 1.8 Hz, benzene); FAB-MS m/z: 356 (M^++1). Anal. Calcd for $C_{13}H_{13}N_3O_5S_2$: C, 43.94; H, 3.69; N, 11.82; S, 18.05. Found: C, 44.11; H, 3.60; N, 11.99; S, 17.85.

5.1.30. *N*-(5-Isopropyl-3-methyl-1,3-thiazol-2(3*H*)-ylidene)-3-nitrobenzenesulfonamide (17g). 76% yield; mp 150–151 °C (isopropanol). ^{1}H NMR (DMSO-d_6) δ: 1.19 (6H, d, J = 6.9 Hz, CH_3 of i-Pr), 2.95 (1H, heptet, J = 6.9 Hz, CH of i-Pr), 3.45 (3H, s, 3-Me), 7.22 (1H, s, thiazole), 7.86 (1H, d, J = 7.8, 8.3 Hz, benzene), 8.26 (1H, br d, J = 7.8 Hz, benzene), 8.43 (1H, ddd, J = 1.0, 1.9, 8.3 Hz, benzene), 8.49 (1H, t, J = 1.9 Hz, benzene); FAB-MS m/z: 342 (M^++1). Anal. Calcd for $C_{13}H_{15}N_3O_4S_2$: C, 45.73; H, 4.43; N, 12.31; S, 18.78. Found: C, 45.66; H, 4.31; N, 12.30; S, 18.89.

5.1.31. *N*-(4-Isobutyl-5-isopropyl-3-methyl-1,3-thiazol-2(3*H*)-ylidene)-3-nitrobenzenesulfonamide (17h). 41% yield; mp 125–126 °C (isopropanol). ^{1}H NMR (DMSO-d_6) δ: 0.90 (6H, d, J = 6.3 Hz, CH_3 of i-Bu), 1.16 (6H, d, J = 6.8 Hz, CH_3 of i-Pr), 1.78 (1H, m, CH of i-Bu), 2.48 (2H, d, J = 7.3 Hz, CH_2 of i-Bu), 3.12 (1H, heptet, J = 6.8 Hz, CH of i-Pr), 3.42 (3H, s, 3-Me), 7.86 (1H, dd, J = 7.8, 8.3 Hz, benzene), 8.23 (1H, br d, J = 7.8 Hz, benzene), 8.42 (1H, ddd, J = 1.0, 2.0, 7.8 Hz, benzene), 8.50 (1H, t, J = 2.0 Hz, benzene);

FAB-MS *m/z*: 398 (M^++1). Anal. Calcd for $C_{17}H_{23}N_3O_4S$: C, 51.36; H, 5.83; N, 10.57; S, 16.13. Found: C, 51.31; H, 5.82; N, 10.62; S, 15.96.

5.1.32. *N*-(5-Isopropyl-3-methyl-4-trifluoromethyl-1,3-thiazol-2(3*H*)-2-ylidene)-3-nitrobenzenesulfonamide (17i). 19% yield; mp 127–128 °C (ethyl acetate–hexane). ^{1}H NMR ($CDCl_3$) δ: 1.31 (6H, d, J = 7.0 Hz, CH_3 of *i*-Pr), 3.53 (1H, m, CH of *i*-Pr), 3.58 (3H, br s, 3-Me), 7.70 (1H, t, J = 7.8 Hz, benzene), 8.30 (1H, dt, J = 2.0, 7.8 Hz, benzene), 8.39 (1H, m, benzene), 8.79 (1H, t, J = 2.0 Hz, benzene); FAB-MS *m/z*: 410 (M^++1). Anal. Calcd for $C_{14}H_{14}F_3N_3O_4S_2$: C, 41.07; H, 3.45; N, 10.26; S, 15.66; F, 13.92. Found: C, 41.02; H, 3.37; N, 10.23; S, 15.64; F, 13.83.

5.1.33. Methyl 5-isopropyl-3-methyl-2-{[(3-nitrophenyl)-sulfonyl]imino}-2,3-dihydro-1,3-thiazole-4-carboxylate (17j). 43% yield; mp 120–121 °C (ethyl acetate–hexane). ^{1}H NMR ($CDCl_3$) δ: 1.29 (6H, d, J = 6.8 Hz, CH_3 of *i*-Pr), 3.67 (3H, s, 3-Me), 3.68 (1H, heptet, J = 6.8 Hz, CH of *i*-Pr), 3.93 (3H, s, CH_3 of COOMe), 7.88 (1H, br t, J = 8.2 Hz, benzene), 8.29 (1H, br d, J = 7.7 Hz, benzene), 8.37 (1H, dd, J = 2.0, 8.2 Hz, benzene), 8.79 (1H, t, J = 2.0 Hz, benzene); FAB-MS *m/z*: 400 (M^++1). Anal. Calcd for $C_{15}H_{17}N_3O_6S_2$: C, 45.10; H, 4.29; N, 10.52; S, 16.06. Found: C, 44.93; H, 4.10; N, 10.62; S, 16.09.

5.1.34. *N*-(4-Chloro-5-isopropyl-3-methyl-1,3-thiazol-2(3*H*)-ylidene)-3-nitrobenzenesulfonamide (17k). 52% yield; mp 140–142 °C (diethyl ether). ^{1}H NMR (DMSO-d_6) δ: 1.20 (6H, d, J = 6.8 Hz, CH_3 of *i*-Pr), 3.18 (1H, heptet, J = 6.8 Hz, CH of *i*-Pr), 3.47 (3H, s, 3-Me), 7.87 (1H, t, J = 7.8 Hz, benzene), 8.28 (1H, br d, J = 7.9 Hz, benzene), 8.45 (1H, br d, J = 7.9 Hz, benzene), 8.50 (1H, br s, benzene); FAB-MS *m/z*: 376 (M^++1). Anal. Calcd for $C_{13}H_{14}N_3O_4S_2Cl$: C, 41.54; H, 3.75; N, 11.18; S, 17.06; Cl, 9.43. Found: C, 41.89; H, 3.74; N, 10.95; S, 16.85; Cl, 9.81.

5.1.35. *N*-(5-*tert*-Butyl-3-ethyl-4-methyl-1,3-thiazol-2(3*H*)-ylidene)-3-nitrobenzenesulfonamide (17l). Compound **17l** was obtained in the same manner as described in the synthesis of **17a** with iodoethane instead of iodomethane. 50% yield; mp 139–141 °C (diethyl ether–hexane). ^{1}H NMR (DMSO-d_6) δ: 1.12 (3H, t, J = 6.8 Hz, CH_3 of Et), 1.32 (9H, s, CH_3 of *t*-Bu), 2.31 (3H, s, 4-Me), 3.96 (2H, q, J = 6.8 Hz, CH_2 of Et), 7.85 (1H, t, J = 8.3 Hz, benzene), 8.25 (1H, ddd, J = 1.0, 1.9, 8.3 Hz, benzene), 8.42 (1H, dd, J = 1.0, 1.9, 8.3 Hz, benzene), 8.48 (1H, t, J = 1.9 Hz, benzene); FAB-MS *m/z*: 384 (M^++1). Anal. Calcd for $C_{16}H_{21}N_3O_4S_2$: C, 50.11; H, 5.52; N, 10.96; S, 16.72. Found: C, 50.06; H, 5.49; N, 11.02; S, 16.81.

5.1.36. *N*-(3,4-Dimethyl-5-vinyl-1,3-thiazol-2(3*H*)-ylidene)-3-nitrobenzenesulfonamide (18). To a solution of **17f** (1.78 g, 5.01 mmol) in methanol (50 mL) was added sodiumborohydride (0.38 g, 10.0 mmol) under ice-bath cooling. The mixture was warmed to room temperature and stirred for 2.5 h. Additional sodiumborohydride (0.19 g, 5.0 mmol) was added and stirred 12 h. The reaction mixture was quenched with aqueous acetone and evaporated. The residue was diluted with ethyl acetate and washed with water and brine, then dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (chloroform–methanol) and recrystallized from isopropanol. To the solution of the product and triethylamine (1.39 mL, 10.0 mmol) in dichloromethane (20 mL) was added methanesulfonyl chloride (0.77 mL, 10.0 mmol) under ice-bath cooling. After the reaction mixture was stirred at the same temperature for 30 min, the solution was warmed to room temperature and stirred for 36 h. The reaction mixture was evaporated and diluted with chloroform. The solution was washed with brine, and then dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (ethyl acetate–hexane) and recrystallized from ethanol to give **18** (459 mg, 21%) as a yellow crystals. Mp 171–173 °C. ^{1}H NMR (DMSO-d_6) δ: 2.27 (3H, s, 4-Me), 3.44 (3H, s, 3-Me), 5.21 (1H, d, J = 10.8 Hz, –CH=CH_2), 5.33 (1H, d, J = 17.0 Hz, –CH=CH_2), 6.89 (1H, dd, J = 10.8, 17.0 Hz, –C*H*=CH_2), 7.86 (1H, t, J = 8.3 Hz, benzene), 8.28 (1H, br d, J = 7.8 Hz, benzene), 8.43 (1H, dd, J = 2.5, 7.8 Hz, benzene), 8.50 (1H, t, J = 2.5 Hz, benzene); FAB-MS *m/z*: 340 (M^++1). Anal. Calcd for $C_{13}H_{13}N_3O_4S_2$: C, 46.01; H, 3.86; N, 12.38; S, 18.90. Found: C, 45.93; H, 3.87; N, 12.10; S, 18.46.

5.1.37. 5-Isopropyl-3-methyl-2-{[(3-nitrophenyl)sulfonyl]imino}-2,3-dihydro-1,3-thiazole-4-carboxamide (19). To a solution of **17j** (877 mg, 2.19 mmol) in tetrahydrofuran (10 mL) was added saturated aqueous ammonia solution (10 mL) and heated to 120 °C in autoclave and stirred for 3 h. The mixture was evaporated in reduced pressure. To the residue was added thionyl chloride (1 mL), *N*,*N*-dimethylformamide (0.15 mL) and stirred at 70 °C for 1 h. Thionyl chloride was removed under reduced pressure and the residue was solved to chloroform (10 mL). To saturated aqueous ammonia (10 mL) was added dropwise the solution under ice-bath cooling and stirred at same temperature for 15 min. The reaction mixture was extracted with ethyl acetate. The organic layer was washed with brine, and then dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue was recrystallized from ethyl acetate–hexane to give **19** (547 mg, 65%) as an orange crystals. Mp 246–248 °C. ^{1}H NMR (DMSO-d_6) δ: 1.20 (6H, d, J = 7.0 Hz, CH_3 of *i*-Pr), 3.31 (1H, m, CH of *i*-Pr), 3.41 (3H, s, 3-Me), 7.87 (1H, t, J = 8.3 Hz, benzene), 8.15 (1H, s, NH_2), 8.19 (1H, s, NH_2), 8.28 (1H, br d, J = 8.3 Hz, benzene), 8.44 (1H, br d, J = 8.3 Hz, benzene), 8.50 (1H, m, benzene); FAB-MS *m/z*: 385 (M^++1). Anal. Calcd for $C_{14}H_{16}N_4O_5S_2$: C, 43.74; H, 4.20; N, 14.57; S, 16.68. Found: C, 43.68; H, 4.35; N, 14.34; S, 16.37.

5.1.38. *N*-(4-Cyano-5-isopropyl-3-methyl-1,3-thiazol-2(3*H*)-ylidene)-3-nitrobenzenesulfonamide (20). To a solution of **19** (268 mg, 0.70 mmol) in 1,2-dichloroethane (5 mL) was added phosphorous oxychloride (0.33 mL, 3.50 mmol) and *N*,*N*-dimethylformamide (0.10 mL) un-

der ice-bath cooling. The mixture was heated to 70 °C and stirred for 1 h. The reaction mixture was pored into ice water and extracted with ethyl acetate. The organic layer was washed with brine, and then dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue was recrystallized from ethyl acetate–hexane to give **20** (180 mg, 70%) as an orange crystals. Mp 136–137 °C. ^{1}H NMR ($CDCl_3$) δ: 1.37 (6H, d, J = 7.0 Hz, CH_3 of *i*-Pr), 3.38 (1H, heptet, J = 7.0 Hz, CH of *i*-Pr), 3.59 (3H, s, 3-Me), 7.70 (1H, t, J = 7.9 Hz, benzene), 8.27 (1H, br d, J = 7.9 Hz, benzene), 8.40 (1H, ddd, J = 2.0, 2.2, 7.9 Hz, benzene), 8.76 (1H, t, J = 2.0 Hz, benzene); FAB-MS *m/z*: 367 (M^++1). Anal. Calcd for $C_{14}H_{14}N_4O_4S_2$: C, 45.89; H, 3.85; N, 15.29; S, 17.50. Found: C, 45.70; H, 3.65; N, 15.32; S, 17.24.

5.2. Pharmacology

5.2.1. Cells and viruses. MT-4 cells[18] and two strains of HIV-1, HIV-1_{IIIB} and HIV-$1_{IIIB\text{-}R}$, were used for the anti-HIV-1 assays. HIV-$1_{IIIB\text{-}R}$, which has a single amino acid change (Y181C) in its RT, is established by serial passages in cell cultures in the presence of escalating concentrations of MKC-442.[19] HIV-$1_{IIIB\text{-}R}$ is also resistant to nevirapine. MT-4 cells were grown and maintained in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), penicillin G (100 units/mL), and gentamicin (20 mg/mL). MT-4 cells and HIV-1_{IIIB} were obtained from Rational Drug Design Laboratories (Fukushima, Japan).

5.2.2. Anti-HIV-1 assay. Determination of the antiviral activity of the test compounds against HIV-1_{IIIB} replication was based on the inhibition of virus-induced cytopathicity in MT-4 cells. Briefly, MT-4 cells were suspended in culture medium at 1×10^5 cells/mL and infected with virus at a multiplicity of infection (MOI) of 0.02. Immediately after virus infection, the cell suspension (100 μL) was brought into each well of a flat-bottomed microtiter tray containing various concentrations of the test compounds. After a 5-day incubation at 37 °C, the number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[20] The HTS of our compound library was also performed using the MTT assay against HIV-$1_{IIIB\text{-}R}$.[19] The anti-HIV-1 activity and cytotoxicity of test compounds were expressed as EC_{50} and CC_{50}, respectively. EC_{50} is the concentration of a test compound that was able to suppress HIV-1 replication by 50%. CC_{50} is the concentration of a test sample that reduced viable cell number by 50% in mock-infected cells. The therapeutic index (TI) is the ratio of CC_{50} to EC_{50}.

5.2.3. In vitro RT inhibition assay. A expression plasmide, pG280, which encodes HIV-1 RT proteins as LacZ fusion proteins were used for the expression of WT RT and mutated RTs.[21] The single amino acid-substituted RTs (K103N RT and Y181C RT) were constructed using pG280 from a Quikchange™ Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA). Recombinant RT enzymes were expressed in *E. coli.* UTX81 and purified by the scheme described by Saitoh et al.[21] In vitro RT assays were conducted according to the previously described method with the following modifications.[22] Test compounds and 0.01 unit of recombinant HIV-1 RT enzymes (either wild type or mutant) were incubated in a reaction mixture (50 μL) containing 50 mM Tris–HCl (pH 8.4), 100 mM KCl, 10 mM $MgCl_2$, 0.1% Triton X-100, 2 mM dithiothreitol, 0.01 OD_{260} of poly(rC)/oligo(dG)$_{12-18}$, and 1 μCi of [1′,2′-^{3}H]dGTP (33 Ci/mmol) at 37 °C for 1 h. The reaction was stopped with 200 μL of 5% cold trichloroacetic acid. The precipitated materials were analyzed for radio activity using a scintillation counter (Aloka Co., Ltd, Tokyo, Japan).

5.2.4. X-ray crystal structure analysis of 17a. A single crystal of **17a** was obtained by recrystallization from MeOH. Crystal data for **17a**: $C_{15}H_{19}N_3O_4S_2$; Mr = 369.45, colorless plate, crystal size 0.45 × 0.18 × 0.02 mm, triclinic, space group P-1, a = 10.1641(6) Å, b = 10.7000(6) Å, c = 9.6632(5) Å, α = 90.408(5)°, β = 106.252(5)°, γ = 63.298(4)°, V = 892.67(9) Å^3, Z = 2, D_{calc} = 1.374 g/cm^3, $F(000)$ = 388, μ(CuKα) = 29.21 cm^{-1}. Data were collected on a RIGAKU AFC5R diffractometer at 298 K. Lattice constants were obtained from a least-squares refinement using the setting angles of 25 reflections carefully centered in the range of 55.34° < 2θ < 62.31°. The structure of **17a** was solved by direct methods using the SIR92 program.[23] The data were corrected for Lorentz and polarization effects. An experimental absorption correction was also applied. All hydrogen atoms were located from difference Fourier synthesis. Full-matrix least-squares refinement was carried out and converged with a final calculated R-factor [$I > 3\sigma(I)$] of 0.043 (Rw = 0.062) and a goodness-of-fit of 1.189.

5.2.5. Docking study. Docking studies for compound **17a** with HIV reverse transcriptase[24] (RT) nonnucleoside binding sites were performed using the GOLD program.[25] Ten independent genetic algorithms (GA) in which a maximum number of 100,000 GA operations were performed on a single population of 100 individuals, were calculated. Operator weights for crossover, mutation, and migration were set to 95, 95, and 10, respectively.

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Review

Potential of 4′-C-substituted nucleosides for the treatment of HIV-1

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Extensive efforts have been made to identify nucleoside reverse transcriptase inhibitors (NRTIs). Eight NRTIs have now been approved for clinical use; however, variants of HIV-1 resistant to these antiviral agents have emerged in patients even when they are treated with combinations [highly active antiretroviral therapy (HAART)]. Thus, the development of novel compounds that are active against drug-resistant HIV-1 variants and that prevent or delay the emergence of resistant HIV-1 variants is urgently needed. Previously, 4′-C-substituted nucleosides (4′-SNs) were designed as new types of NRTIs. They were synthesized and examined as potential therapeutic agents against HIV infection. Among them, several 4′-substituted-2′-deoxynucleosides (4′-SdNs), especially those that bear an ethynyl group, were shown to be active against various laboratory and clinical HIV-1 strains including known drug-resistant variants. These results were recently reported by our collaborators. In this review, we summarize the design, synthesis and demonstrations of the anti-HIV activity of 4′-SNs, and then consider 4′-SNs as potential therapeutic agents for HIV-1.

Keywords: NRTIs, 4′-SNs, anti-HIV-1 agents, HAART, drug-resistant HIV-1 variants

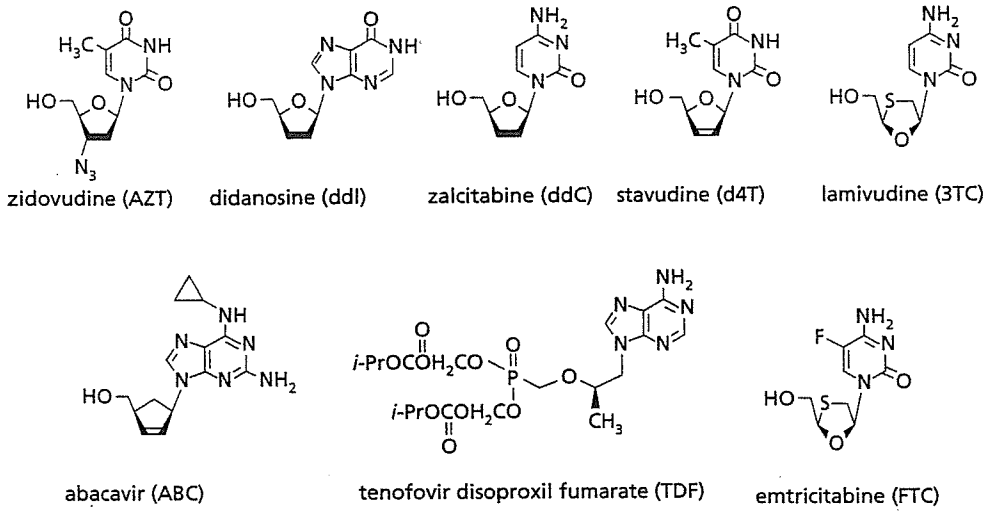
Introduction

The development of novel antiviral agents is essential in the battle against viruses such as HIV, because drug-resistant variants emerge. For the treatment of acquired immunodeficiency syndrome (AIDS), eight NRTIs have been approved for clinical use to date: 3′-azido-3′-deoxythymidine [zidovudine (AZT)], 2′,3′-dideoxyinosine [didanosine (ddI)], 2′,3′-dideoxycytdine [zalcitabine (ddC)], 2′,3′-dehydro-2′,3′-dideoxythymidine [stavudine (d4T)], L-1,3-oxathiolanylcytosine [lamivudine (3TC)], abacavir (ABC), tenofovir disoproxil fumarate (TDF), and L-1,3-oxathiolanyl-5-fluorocytosin [emtricitabine (FTC)] (Figure 1).

HAART using two, or more, NRTIs and protease inhibitors (PIs) has dramatically improved the quality of life and survival of patients infected with HIV-1. But the emergence of drug-resistant mutants has been a critical problem in using these chemotherapeutic agents; furthermore, some of these mutants show high levels of cross-resistance. Consequently, the development of structurally new nucleoside derivatives that are active against HIV-1 variants resistant to the existing 2′,3′-dideoxy nucleosides is urgently needed.

There are six classes of chemotherapeutic agent against HIV-1 so far: 1) NRTIs, mentioned above; 2) non-nucleoside reverse transcriptase inhibitors (NNRTIs); 3) protease inhibitors (PIs); 4) integrase inhibitors (INIs); 5) fusion inhibitors (FIs) and 6) chemokine receptor antagonists (CRAs). A number of NRTIs, NNRTIs and PIs are currently used clinically. Much progress has been made in the classes of FIs and CRAs, but INIs are still in the pre-clinical stage.

Figure 1. Structures of clinically used nucleoside analogues as NRTIs



During our exploration of novel NRTIs, we recently designed and synthesized a series of 4′-SdNs derivatives. Among these, 4′-*C*-ethynyl-2′-deoxynucleosides (4′-EdNs) showed promising features in both their biological activities and their structures (Kodama *et al.*, 2001). They inhibited the replication of multidrug-resistant clinical HIV-1 strains carrying a wide variety of drug resistance-related amino acid substitutions isolated from HIV-1-infected individuals, for whom 10 or 11 different anti-HIV-1 agents had failed. These 4′-EdNs have a 2′-deoxyribose moiety, unlike all of the currently available NRTIs. Additionally, all of these 4′-EdNs blocked the replication of a wide spectrum of laboratory and clinical HIV-1 strains *in vitro* with low cellular toxicities. Therefore, we set out to search for promising new candidates.

Synthesis and anti-HIV activity of 4′-SNs

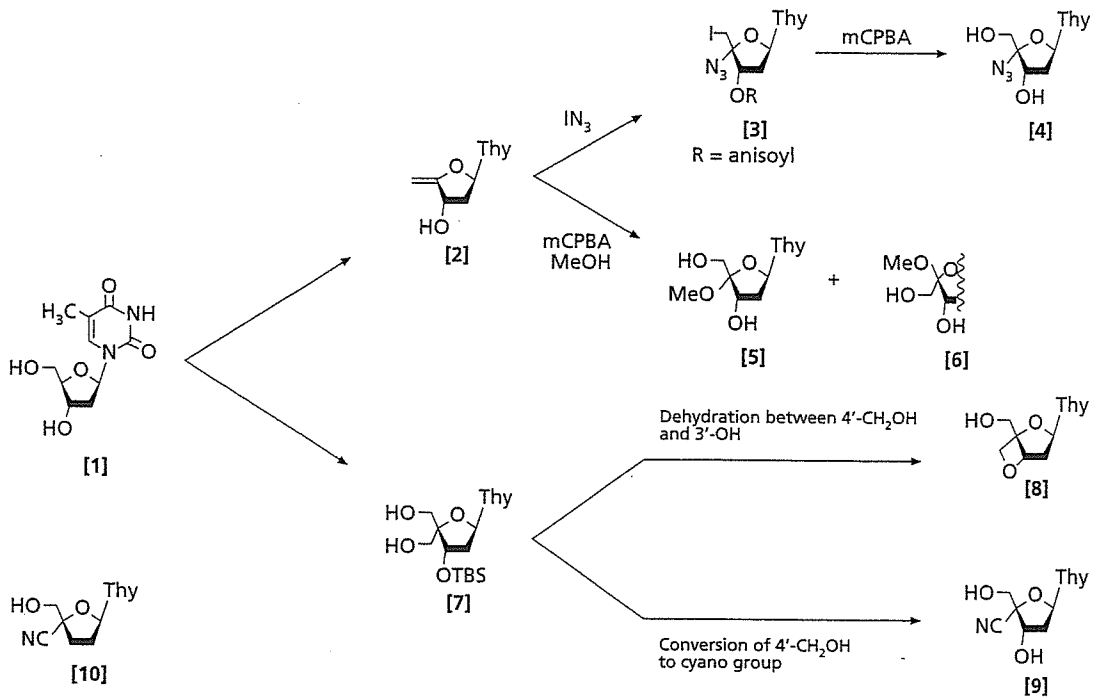
4′-Substituted nucleosides have been under development since JG Moffatt's group accomplished the synthesis of 4′-*C*-fluoro-5′-*O*-sulphamoyladenosine, the antibiotic nucleocidin and related nucleosides (Verheyden *et al.*, 1975, Jenkins *et al.*, 1976, Owen *et al.*, 1976, Youssefyeh *et al.*, 1977 & 1979, Jones *et al.*, 1979).

Compared with 2′- and 3′-substituted nucleoside derivatives, methods for the synthesis of 4′-SNs were very difficult. However, since the 1990s, several research groups have attempted the synthesis of 4′-SNs and the results, including biological activities, published.

Initially, the Syntex group, led by JG Moffat, pioneered the exploration for an improved method (Figure 2). Maag *et al.* reported the synthesis and anti-HIV activity of 4′-*C*-azidothymidine (4′-AZT 4) and 4′-*C*-methoxynucleosides (**5**) (Maag *et al.*, 1992). The key steps in the synthesis of the 4′-azido analogues were the stereo- and regioselective addition of iodine azido to a 4′,5′-unsaturated nucleoside precursor **[2]** followed by an oxidatively assisted displacement of the 5′-iodo group. 4′-AZT **[4]** led to potent activity against HIV-1 *in vitro*, especially its activity against HIV mutants which were resistant to AZT. IC_{50} was 0.01 μM against HIV-1 (LAV-IIIb) replication in A301 cells. Structure–activity relationships among HIV inhibitory 4′-*C*-substituted nucleosides were published by the Syntex research group (Prisbe *et al.*, 1993).

O-Yang, of Syntex, also reported two interesting findings: that 1) the fused oxetane derivative of thymidine **[8]** inhibited HIV replication in A301 cells with remarkably low bone marrow toxicity (O-Yang *et al.*, 1992); and

Figure 2. Synthesis of various 4′-SNs by Syntex research groups



2) 4′-*C*-cyanothymidine (4′-CNT **[9]**) inhibited HIV in A301 cells with an IC_{50} of 0.002 μM (O-Yang *et al.*, 1992). 4′-*C*-cyano-3′-deoxythymidine **[10]** was also synthesized from 3′-deoxythymidine, but it was not active against HIV (O-Yang *et al.*, 1992). Additionally, both oxetane fused **[8]** and 4′-*C*-cyano **[9]** derivatives were prepared via similar intermediates **[7]** bearing a hydroxymethyl group at the *C*-4′ position of the sugar moiety.

Subsequently, Chen and colleagues reported the mechanism of action of 4′-AZT **[4]** against HIV-1 to be through its DNA chain-terminating activity (Chen *et al.*, 1993).

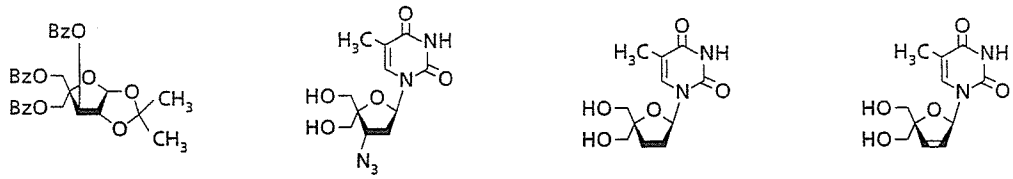
Results similar to those of the Syntex research group were published by A Holy's group (Hrebabecky *et al.*, 1993). They reported the synthesis of 4′α-*C*-hydroxymethyl thymidine derivatives of AZT **[12]**, ddT **[13]** and d4T **[14]** related Ns starting from 1,2-*O*-isopropylidene-3,5-di-*O*-benzoyl-4-*C*-benzoyloxymethyl-β-L-arabinofuranose **[11]** (Figure 3).

JG Moffatt's group introduced a hydroxymethyl group at the 4′α-position of nucleosides using the Cannizzaro reaction 25 years ago, using an appropriately protected ribose 5-aldehyde **[15]** (Figure 4) (Youssefyeh *et al.*, 1979).

Since oxetane-fused derivatives of thymidine **[8]** and 4′-CNT **[9]** showed potent anti-HIV activity as mentioned in the Syntex report, A Matsuda's group reported that they adopted Moffatt's method to synthesize their target nucleosides via 4′-*C*-formyl derivatives **[19]**: 4′-*C*-ethynyl, -vinyl, -ethyl, -chlorovinyl, -cyano, and -methyl derivatives of pyrimidine nucleosides **[20–28]** (Figure 5) (Nomura *et al.*, 1999), (Sugimoto *et al.*, 1999). Moreover, they have recently reported the synthesis of 4′-*C*-branched thymidine **[31–34]** by the use of an intramolecular radical cyclization reaction (Figure 5) (Sugimoto *et al.*, 1999). They showed that 4′-*C*-substituted thymidine **[31–34]** including 4′-ET **[28]** exhibited potent activity against not only HIV-1 but also herpes simplex type 1. Besides the foregoing, the biological activities of these derivatives have also been reported in a 2′-deoxycytidine, cytidine and uridine series (Nomura *et al.*, 1999).

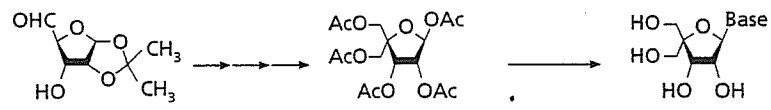
A new synthetic method leading to a series of 4′-*C*-branched 2′,3′-didehydro-2′,3′-dideoxyuridine (4′-Sd4U

Figure 3. Structures of 4-hydroxymethyl sugar **[11]** and nucleosides **[12–14]**



[11] [12] [13] [14]

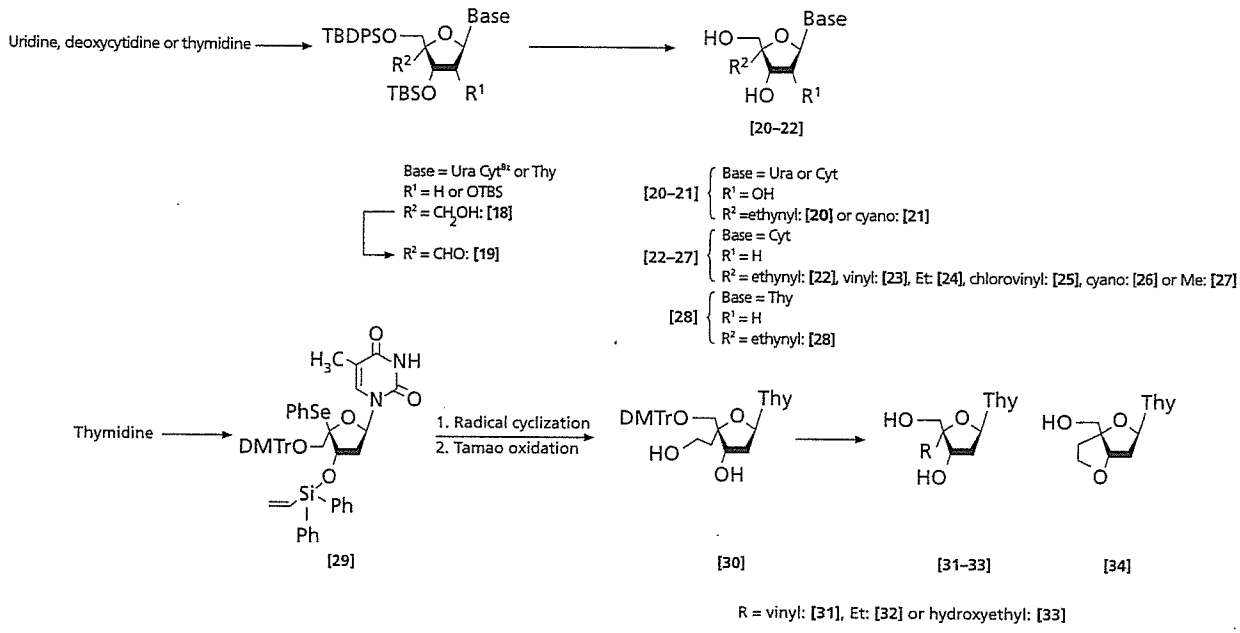
Figure 4. Synthesis of 4′-C-hydroxymethyl nucleosides by Cannizzaro reaction aldehyde **[15]** with formaldehyde



[15] [16] [17]

[36–42] was developed (Haraguchi *et al.*, 1992). This method was based on $SnCl_4$-promoted allylic rearrangement to give 4′-Sd4U **[36–42]** (Figure 6). However, they did not mention the biological activities of these derivatives. H Tanaka's group also very recently published a paper describing the synthesis and activity of 2′,3′-didehydro-3′-deoxy-4′-ethynyl-thymidine (4′-Ed4T) **[47]** (Haraguchi *et al.*, 2003). The key step of this reaction consisted of the ring-opening of 4′,5′-epoxy precursors **[43]** with aluminium reagents resulting in the formation of 4′-*C*-substituted nucleoside analogues **[45–47]** (Figure 6). In this reaction, the 3′-configuration of 4′,5′-epoxide **[43]** was very important to form 4′-SNs having the expected 4′-configuration. Very interestingly, 4′-Ed4T **[47]** was active against HIV-1 with an EC_{50} value of 0.20 μM, which was 14-fold more potent than that of d4T (EC_{50}=2.8 μM); 4′-Ed4T's cytotoxicity was low in comparison. In order to determine SAR, H Tanaka's group went on to prepare 4′-*C*-cyano-2′,3′-didehydro-3′-deoxythymidine (4′-CNd4T) **[42]** (Haraguchi *et al.*, 2003) by allylic substitution of the

Figure 5. Synthesis of 4′-*C*-substituted nucleosides by conversion of 4′-*C*-formyl derivatives and by radical cyclization reaction



3′,4′-unsaturated nucleoside **[35]**, having a leaving group at the 2′-position, with cyanotrimethylsilane in the presence of stannic chloride ($SnCl_4$) (Figure 6). Unfortunately, 4′-CNd4T **[42]**' s activity was only one-fifth that of d4T. One of their derivatives, 4′-Ed4T **[47]**, is expected to become a promising new NRTI candidate

4′-Trifluoromethylthymidine derivatives **[52, 53, 55]** and related purine nucleosides **[54, 56, 57]** were synthesized by Johnson (Figure 7) (Johnson *et al.* 1998). A strategy based on the use of (trifluoromethyl)trimethylsilane for introduction of a trifluoromethyl group at the *C*-4 of ribose was developed. Unfortunately, these nucleosides were not active against HIV.

Compared to 4′-*C*-substituted nucleosides, there are few reports on the synthesis of 4′α-carbon substituted carbocyclic nucleosides, the most common method being transformation from a natural product. The functionalization of the cyclopentene moiety is restricted in these cases. Interestingly, Kato reported that enantio- and diastereoselective synthesis of 4′-α-alkylcarbovir derivatives was achieved based on Sakai's asymmetric alkylation of β-keto esters (Kato *et al.*, 1998). This method and the related papers cited in his report will enable us to make many carobocyclic derivatives.

For the readers' reference, we cite related reports known to us for the synthesis of various 4′-*C*-substituted nucleosides: (Secrist III *et al.*, 1978; Johnson *et al.*, 1994; Thrane *et al.*, 1995; Marx *et al.*, 1996; Wang *et al.*, 1996; Kozak *et al.*, 1998; Singh *et al.*, 1998; Imanishi *et al.*, 1998; Wang *et al.*, 1999; Crich *et al.*, 1999; Jung *et al.*, 2001; Summerer *et al.*, 2001).

Chemistry and biological activity of 4′-SNs

Two principle methods were employed for the preparation of 4′-SNs: 1) condensation and 2) modification starting from natural nucleosides. The first approach used for the preparation of 4′-SNs was the condensation method; this is an efficient route to various derivatives. Modification starting from natural nucleosides readily scaled up, creating several candidates.

Therefore, initially we started our chemistry by the condensation method to explore the seeds, and then we utilized

Figure 6. Synthesis of 4′-*C*-substituted nucleosides by $SnCl_4$-promoted allylic rearrangement reaction and by ring-opening reaction of 4′,5′-epoxy nucleosides **[43]**

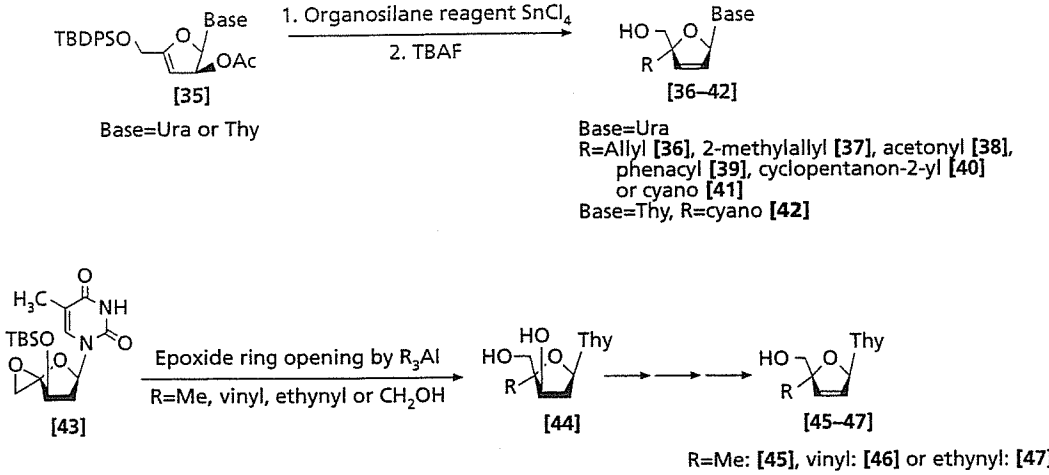
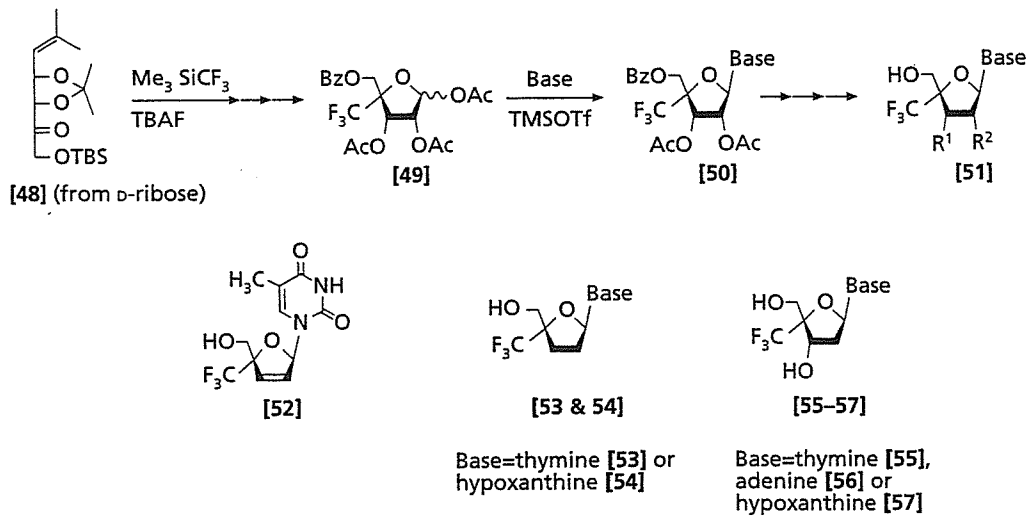


Figure 7. Synthesis of 4′-*C*-trifluoromethyl nucleosides **[52–57]**



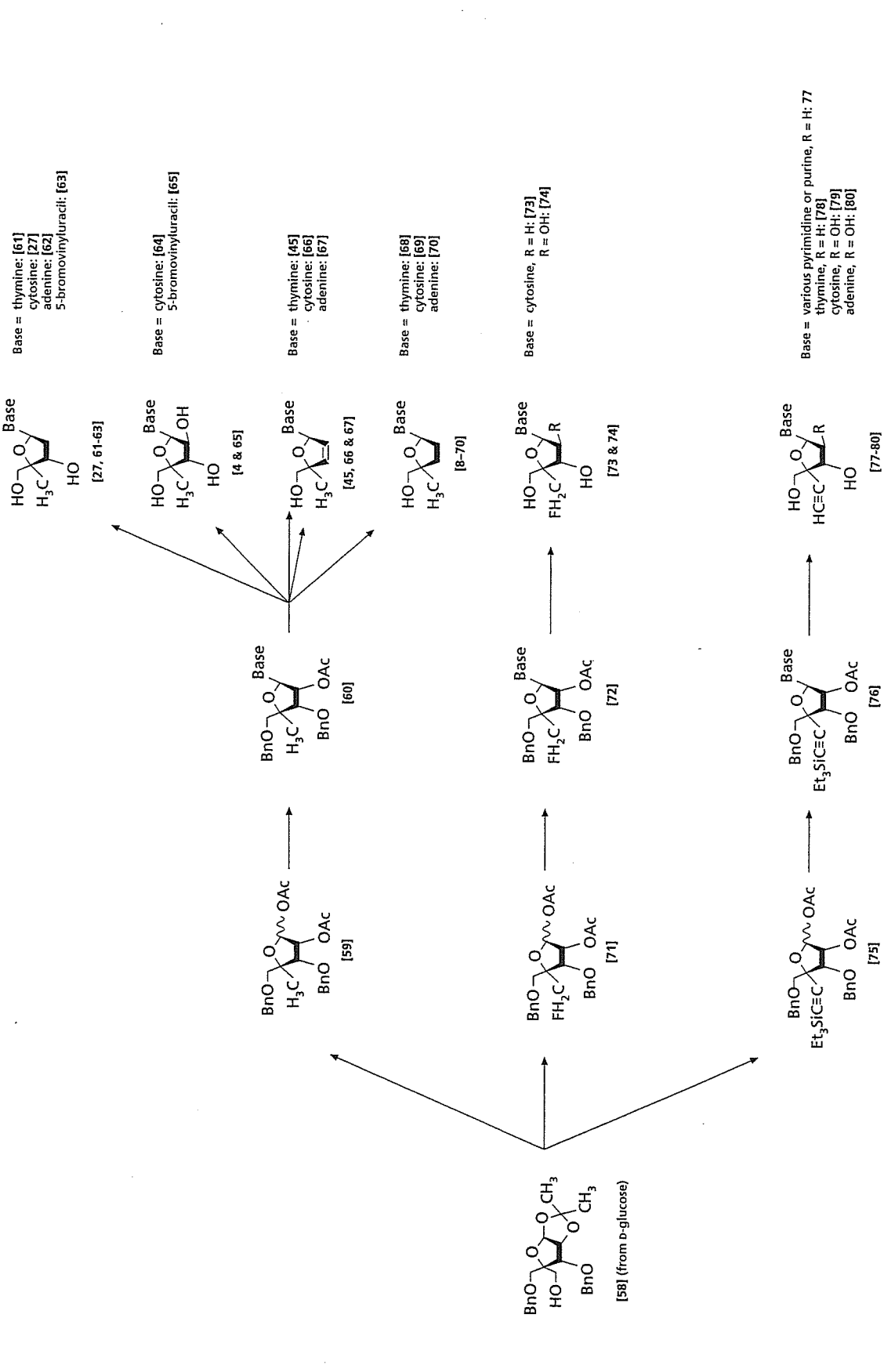


Figure 8. Synthesis of 4′-C-methyl, fluoromethyl and ethynyl nucleosides by condensation of sugars with bases

both methods depending on the structure of the target derivatives. We summarize our synthesis of 4′-*C*-methyl, fluoromethyl and ethynyl nucleosides using the condensation method (Figure 8). We started our chemistry with the synthesis of 4′-*C*-methyl nucleosides (Ohrui *et al.*, 1991). These 4′-SNs **[27, 45, 61–70]** were prepared by the condensation method, which utilized a key intermediate, 4-*C*-methyl-D-ribofuranose derivative **[59]** (Waga *et al.*, 1993). During the preparation of a series of 2′-deoxynucleoside **[27, 61, 62]**, 2′,3′-unsaturated nucleoside **[45, 66, 67]**, 2′,3′-dideoxynucleoside **[68–70]** and ara-*C* analogues **[64]** by the above method, we found that 4′-*C*-methyl-2′-deoxycytidine (4′-MdC) **[27]** showed inhibitory activity against HIV in MT-4 cells (Waga *et al.*, 1996).

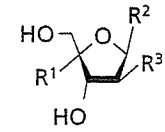
Although 4′-MdC **[27]** was 100-fold more potent than the thymidine derivative 4′-MT **[61]**, it was the most cytotoxic compound among the 4′-*C*-methyl nucleosides tested. Interestingly, the 4′-MdC **[27]** was also tested for its ability to inhibit the growth of P388 mouse leukaemia cells, and it proved to be markedly effective (IC_{50}=1.7 μM). The mechanism of action of **[27]** was also studied (Yamaguchi *et al.*, 1997).

Since the Yamasa Corporation developed BVaraU as an anti-HSV-1 drug, we prepared 4′-*C*-methyl-BVaraU **[65]** and 4′-*C*-methyl-BVDU **[63]** as antiviral agents. Compound **[63]** exhibited particularly potent anti-HSV-1 and anti-varicella-zoster virus (VZV) activity (Kitano *et al.*, 1999). Additionally, 4′-*C*-fluoromethyl nucleosides such as 2-deoxy-D-erythro- and *arabino*-pentophuranosyl cytosine **[73,74]** were previously synthesized by us using an analogous method (Kitano *et al.*, 1997). Interestingly, 4′-*C*-fluoromethyl-2′-deoxycytidine (4′-FMdC) **[73]** exhibited not only potent anti-HIV activity but also anti-neoplastic activity.

As mentioned above, 4′-ethynyl-pyrimidine-nucleosides showed anti-HIV activity. However, there were only a few reports on the preparation of 4′-ENs. Moreover, those papers did not mention any synthetic method of 4′-ENs, especially in the case of purine nucleosides. Thus, we started the chemistry for modifying the 4′-*C*-position of purine nucleosides with an ethynyl group. 4′-ENs could be prepared from the corresponding nucleosides, but we usually used 4-*C*-hydroxymethyl-3,5-di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-*ribo*-furanose **[75]** as a versatile starting material for the synthesis of D-arabino and 2′-deoxy-D-ribo analogues of 4′-ENs **[77–80]**. The outline of our study for the synthesis of 4′-ENs is shown in Figure 8 (Kohgo *et al.*, 1999), and the anti-HIV activity is summarized in Figure 9 and Table 1 (Maag *et al.*, 1992; O-Yang *et al.*, 1999; Nomura *et al.*, 1999; Sugimoto *et al.*, 1999; Ohrui *et al.*, 2000; Ohri *et al.*, 2001).

Since 4′-*C*-substituted nucleosides showed anti-HIV activity (Table 1), we decided to explore the novel NRTIs that are active against drug-resistant HIV-1 variants and that prevent or delay the emergence of resistant HIV-1.

Figure 9. Structures of 4′-*C*-substituted nucleosides*



*See Table 1 for anti-HIV activity of R^1, R^2 and R^3.

Summary of SARs of 4′-SNs against HIV-1

As described in other research groups' investigations, the syntheses and anti-HIV activity of 4′-*C*-methyl-thymidine (4′-MT) **[61]**, 4′-*C*-ethynyl-2′-deoxycytidine (4′-EdC) **[22]**, 4′-*C*-ethyl-2′-deoxycytidine (4′-EtdC) **[24]**, 4′-*C*-ethynyl-thymidine (4′-ET) **[28]** and some other 4′-SdNs were reported while we were working on our project. Therefore, SAR of various 4′-*C*-substituted nucleosides against HIV-1 are summarized together with our data:

1) The estimated relative order of anti-HIV-1 potency is as follows: $CN \geq N_3 \geq C{\equiv}CH > CH{\equiv}CH_2 > Me{=}Et > C{\equiv}C$–Me. This is based on published data. Interestingly, the order is the reverse of the $-\Delta G^0$ values between equatorial and axial substituents on a cyclohexane ring: $CN < F < C{\equiv}CH < CH{=}CH_2 < Me \leq Et < t$-Bu. Thus, these results indicate that the structure of 4′-SNs with a less sterically demanding substituent at the 4′-position is closer to the structure of dNs and has greater anti-HIV activity.

2) Purine analogues are generally less toxic than pyrimidine analogues; the former generally have greater selectivity indices (SI).

3) Ribo-derivatives are inactive, and arabino-derivatives are inactive or weakly active compared with 2′-deoxyribo counterparts.

4) 2′,3′-Dideoxyribo derivatives, including d4 type derivatives, with some exceptions do not show anti-HIV activity.

Design for creating novel NRTIs

One of our collaborators (Ohrui, 2001) speculated that the expected chemical and biological mechanisms of novel NRTIs were as follows:

1) The expected properties come from the presence of a 3′α-OH group.

Table 1. Anti-HIV activity of various 4′-*C*-substituted pyrimidine and purine nucleosides

Compound			Anti-HIV activity		
R^1	R^2	R^3	EC_{50} (μM)	CC_{50} (μM)	SI
CN	thymine	H	0.002	1	500
N_3	thymine	H	0.01	8	800
ethynyl	thymine	H	0.61	>380	>623
ethynyl	thymine	OH	>350	>350	–
ethynyl	5-ethyluracil	H	>360	>360	–
ethynyl	uracil	H	>100	>100	–
ethynyl	5-fluorouracil	H	>10	3.4	–
ethynyl	5-chlorouracil	H	6.0	81.7	13.6
ethynyl	5-bromouracil	H	2.3	>100	>43.5
ethynyl	5-iodouracil	H	0.34	>260	>765
CH_2N_3	thymine	H	2.1	333	159
Me	thymine	H	7.2	104	14.4
Et	thymine	H	16.1	>100	6.21
OMe	thymine	H	8.49	200	23.6
vinyl	thymine	H	6.1	>100	>16.4
hydroxyethyl	thymine	H	>4.7	4.7	–
propynyl	thymine	H	>100	>100	–
CN	cytosine	H	0.0012	0.17	142
N_3	cytosine	H	0.01	8	800
ethynyl	cytosine	H	0.0048	0.92	192
ethynyl	cytosine	OH	0.0048	1.74	363
ethynyl	5-methylcytosine	H	0.011	0.70	63
ethynyl	5-fluorocytosine	H	0.030	>100	>3333
ethynyl	5-chlorocytosine	H	>100	>100	–
ethynyl	5-bromocytosine	H	>100	>100	–
ethynyl	5-iodocytosine	H	>100	>100	–
Me	cytosine	H	0.015	1.0	66.7
CH_2F	cytosine	H	0.0068	0.12	18
Et	cytosine	H	0.013	0.77	59
vinyl	cytosine	H	0.0086	0.18	21
chlorovinyl	cytosine	H	2.1	4.6	2.2
N_3	adenine	H	0.13	50	385
ethynyl	adenine	H	0.098	16	1630
ethynyl	adenine	OH	0.78	248	318
ethynyl	2,6-diaminopurine	H	0.00034	0.9	2600
ethynyl	hypoxanthine	H	0.13	137	1053
ethynyl	guanine	H	0.0015	1.4	933
ethynyl	purine	H	135	>400	>3
methyl	adenine	H	2.6	2.6	–
	zidovudine (AZT)		0.0032	29.4	9190
	lamivudine (3TC)		0.10	>100	933

Figure 10. Speculations on how to overcome NRTIs' problems

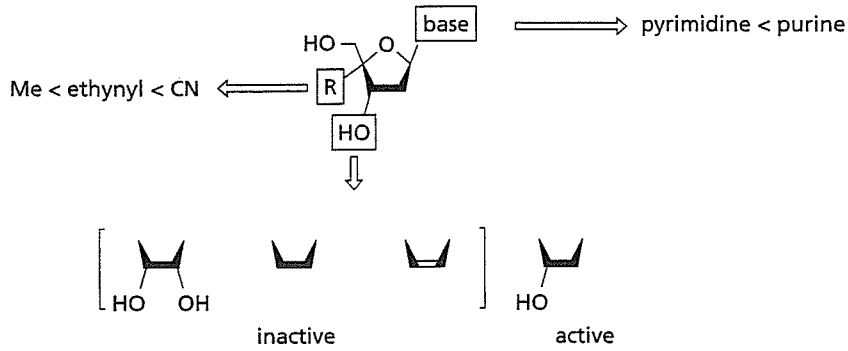
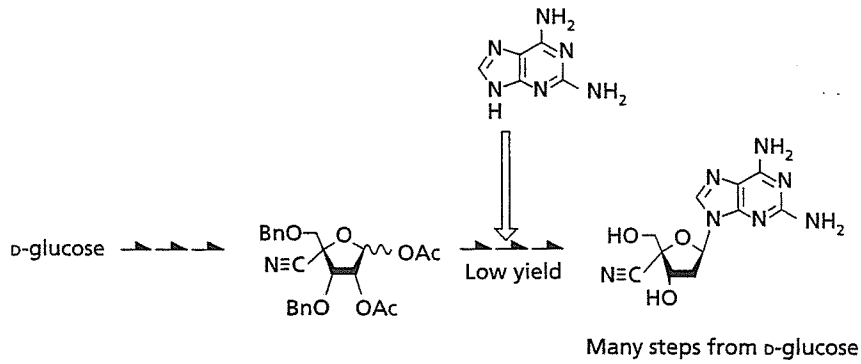


Figure 11. Synthetic problems of 4′-C-substituted nucleosides by condensation method



2) The presence of 3′α-OH in 4′-SdNs makes it acceptable by RTs and, therefore, 4′-SdNs are incorporated into the proviral DNA chains. The electron-withdrawing 3′α-OH group makes 4′-SdNs acid-stable even with purines. Thus, various purine derivatives can be made.

3) The expected properties come from the presence of 4′-substituents.

4) The 4′-substituents cause severe steric hindrance to the neighbouring *cis* 3′α-OH group due to restricted rotation around the C3′-C4′ single bond. Thus, the reactivity of 3′α-OH sharply decreases. Therefore, it was expected that enzymatic chain elongation of DNA would not proceed by using the very unreactive 3′α-OH. Consequently, 4′-SdNs could be chain terminators for proviral DNA biosynthesis. The steric repulsion between 3′α-OH and 4′-substituents changes the conformation of the furanose ring of 4′-SdNs, preferably to 3′-endo conformation (N-Type); this results in 4′-SdNs being less susceptible to enzymatic degradation. Therefore, 4′-SdNs would be more stable than 2′-deoxynucleosides (dN) and 2′,3′-dideoxynucleosides (ddN) against catabolism. The lipophilic substituent at the 4′-position imparts more lipophilicity to 4′-SdNs thus enabling them to penetrate the cell membrane efficiently. Possibly, this may enhance oral bioavailability and penetration through the